Effect of Thalamocortical Activation on Recruiting Responses

III. Reticular Lesions

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IN PREVIOUS studies [21, 25] thalamocortical activation was evaluated by blocking of the cortical recruiting responses. Thalamocortical activation was induced by high frequency stimulation of the reticular formation and other central structures as well as by activation of different sensory modalities. In all cases, thalamocortical activation was similar and consisted in a generalized suppression of the amplitude modulation of the cortical recruiting responses (waxing and waning). It was postulated that electrocortical activation is a unitary function mediated by a common reticular mechanism.

The present study investigates the effect of acute mesencephalic reticular lesions on thalamocortical activation induced by central and peripheral stimulation. In addition, it investigates the nature of the tonic reticular influences on thalamocortical systems in the intact waking animal.

Method

Experiments were carried out on twenty cats immobilized with Flaxedil (gallamine triethiodide), protected by local anesthesia. Cortical recruiting responses were elicited by repetitive 8/sec stimulation of centrum medianum (CM), N. centralis medialis (NCM) and N. centralis lateralis (CL). Blocking of recruiting was produced by high frequency stimulation of the frontal cortex, basolateral amygdala, head of the caudate nucleus, N. centrum medianum and cerebellar cortex. Also, visual, olfactory, auditory, proprioceptive and nociceptive stimulation was utilized to block recruiting. Methods employed in the production of recruiting responses as well as in the blocking of recruiting were identical to those reported in a previous paper.[21]

Recruiting and blocking of recruiting were compared in the intact animal and following mesencephalic reticular lesions.

* This work was supported by U.S. Navy contracts Nonr-233(32) and Nonr-4756; assistance was also provided by the Mental Health Training Program (USPHS grant MH-6415) and NASA grant NGL 05-007-049.

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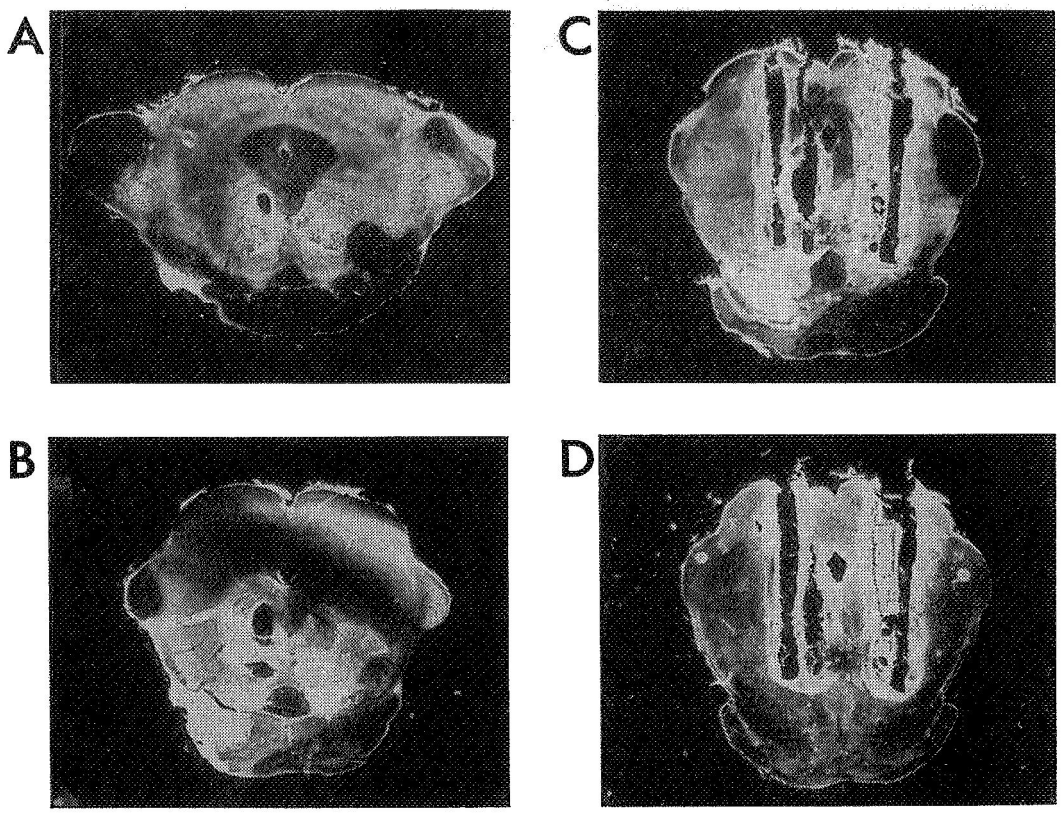

FIG. 1.— *Serial sections of the brain stem showing typical extent of the reticular lesions.* A, B, C and D, lesions involving the mesencephalic tegmentum and the inferior part of the periaqueductal grey at the level of the superior colliculi (F 3.5 to F 1).

Electrolytic lesions were produced through eight parallel electrodes, stereotaxically inserted into the mesencephalic tegmentum. Electrodes were fine gauge stainless steel probes, insulated except for 2 mm at the tip. Each individual lesion was made by passing 4 mA of anodal current for 60 secs. The total area of electrocoagulation, involving 24 individual lesions, was approximately 8 mm^2.

At the conclusion of the experiments, the loci of the stimulating electrodes were grossly delineated. Reconstructions of reticular lesions were made by serial 30 μ sections, stained by Weil and thionin techniques.

Results

Reticular Lesions

Figure 1 shows the nature and extent of the reticular lesions. Lesions involved the mesencephalic tegmentum and the inferior part of the periaqueductual grey matter at the level of the superior colliculi. In 15 cases, both tectum and specific ascending pathways were left intact. The lesions did not spread more anteriorly than to frontal plane 3.5.

Suitable preparations fulfilled the following criteria: (a) pupils symmetrical and myotic, (b) symmetrical spasticity of the hind limbs, (c) depression of the trigeminal reflexes, (d) presence of evoked potentials in the sensory motor cortex after single shock applied to the sciatic nerve, (e) EEG background activity consisting of periodic spontaneous spindle bursts.

Effect of Reticular Lesions on Recruiting Responses

The low-voltage, fast, electrocortical activity of an intact flaxedilized animal (Fig. 2A) was replaced by recurring bursts of spindling activity immediately after reticular lesions (Fig. 2A'). Cortical spindle bursts were formed by 8-12 c/sec waves which were widespread, symmetrical and with maximal amplitude in cortical association areas.

Repetitive (8/sec) thalamic stimulation evoked cortical recruiting responses which displayed marked differences before and after reticular lesions. In the intact animal, cortical recruiting responses showed an initial incrementation, but with relatively constant amplitude and lack of modulation (waxing and waning) to continued stimulation (Fig. 2B). In contrast, after reticular lesions the same thalamic stimulation induced cortical recruiting responses which showed marked periodic amplitude modulation (Fig. 2B'). This waxing and waning continued as long as the stimulation was applied and seemed to follow the general pattern of spontaneous spindle bursts.

Other differences in cortical recruiting responses before and after the reticular lesions were the following.

Low Recruiting Threshold.

After reticular lesions recruiting threshold was lower than in the intact animal. Differences in threshold were most clearly observed when the controls were obtained upon a desynchronized background activity and when recruiting was initiated by stimulation of N. centralis lateralis (CL) and N. centrum medianum (CM). Threshold for recruiting after reticular lesions in this case was 2.5 volts lower than in the intact animal. No difference in recruiting threshold was found when control recruiting in the intact animal was elicited under optimal synchronization of the background activity and by stimulation of N. centralis medialis (NCM).

Cortical Responses to Single Shock Thalamic Stimulation.

In the intact animal a single shock applied to the thalamus failed to produce a response or gave a short, circumscribed unilateral cortical afterdischarge formed by 8-12 c/sec rhythmic waves. Following reticular lesions similar thalamic stimulation produced large and widespread, predominantly bifrontal, cortical afterdischarge (Fig. 3). This cortical response had a constant latency of 100-200 msec in cortical areas ipsilateral to the site of thalamic stimulation.

The duration and amplitude of the cortical afterdischarge was, however,

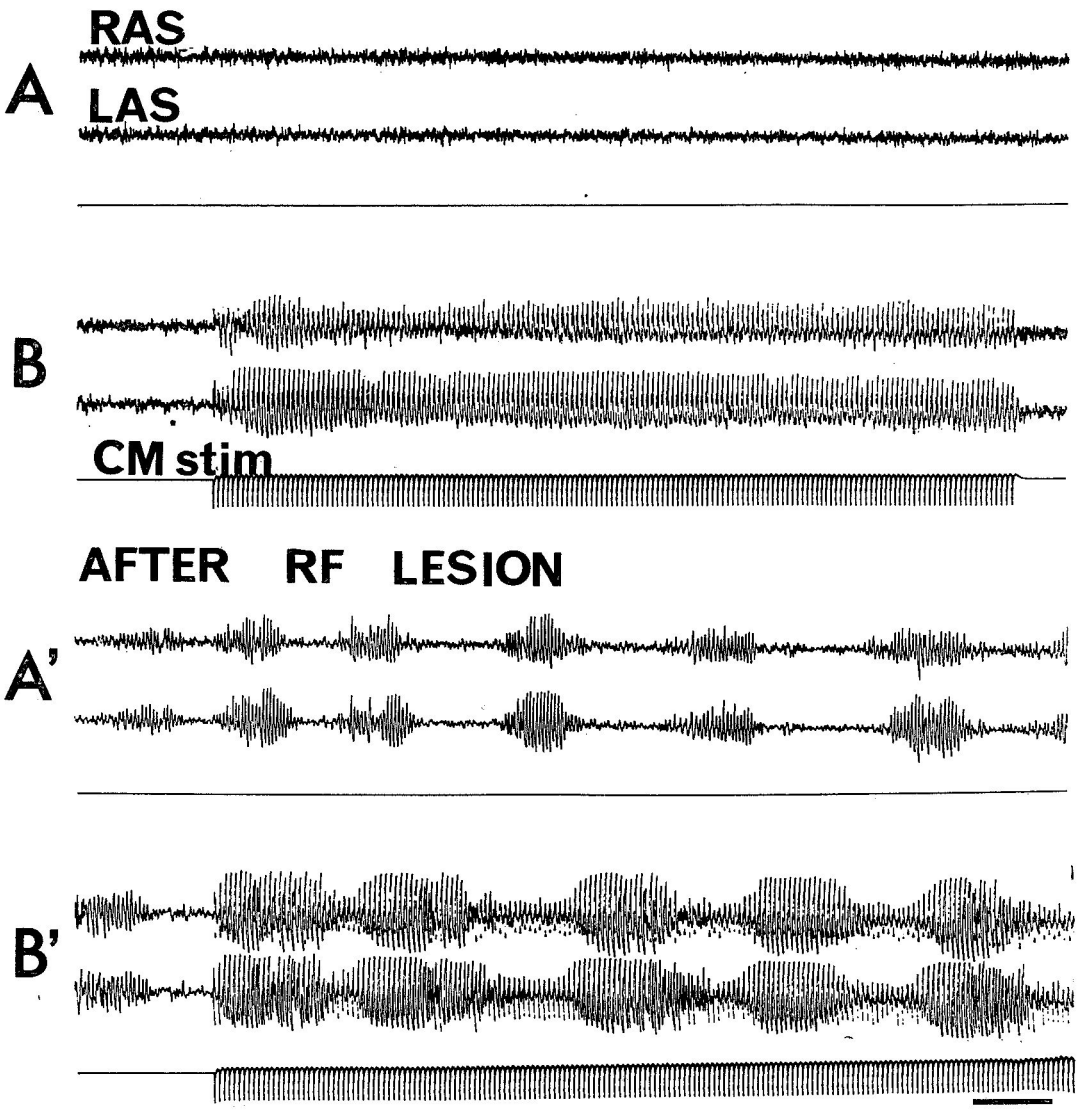

FIG. 2.— *Effect of reticular lesions on recruiting responses.* Electrical recordings from the right anterior sigmoid gyrus (upper trace) and left anterior sigmoid gyrus (lower trace). A, spontaneous desynchronized electrical activity in the intact animal. B, cortical recruiting responses elicited by stimulation of N. centrum medianum (8/sec, 3.V) in the intact animal. A', spontaneous synchronized electrical activity showing spindle bursts after reticular lesions. B', cortical recruiting responses as in B, following reticular lesions. Calibration, 2 sec.

variable and depended on the ocurrence of the spontaneous spindle bursts. Maximal afterdischarge was elicited when the thalamic stimulus was applied 2-3 sec after the end of a spontaneous spindle. In contrast, minimal afterdischarge was observed when the stimulus was presented at the onset or in the middle of a spontaneous spindle burst.

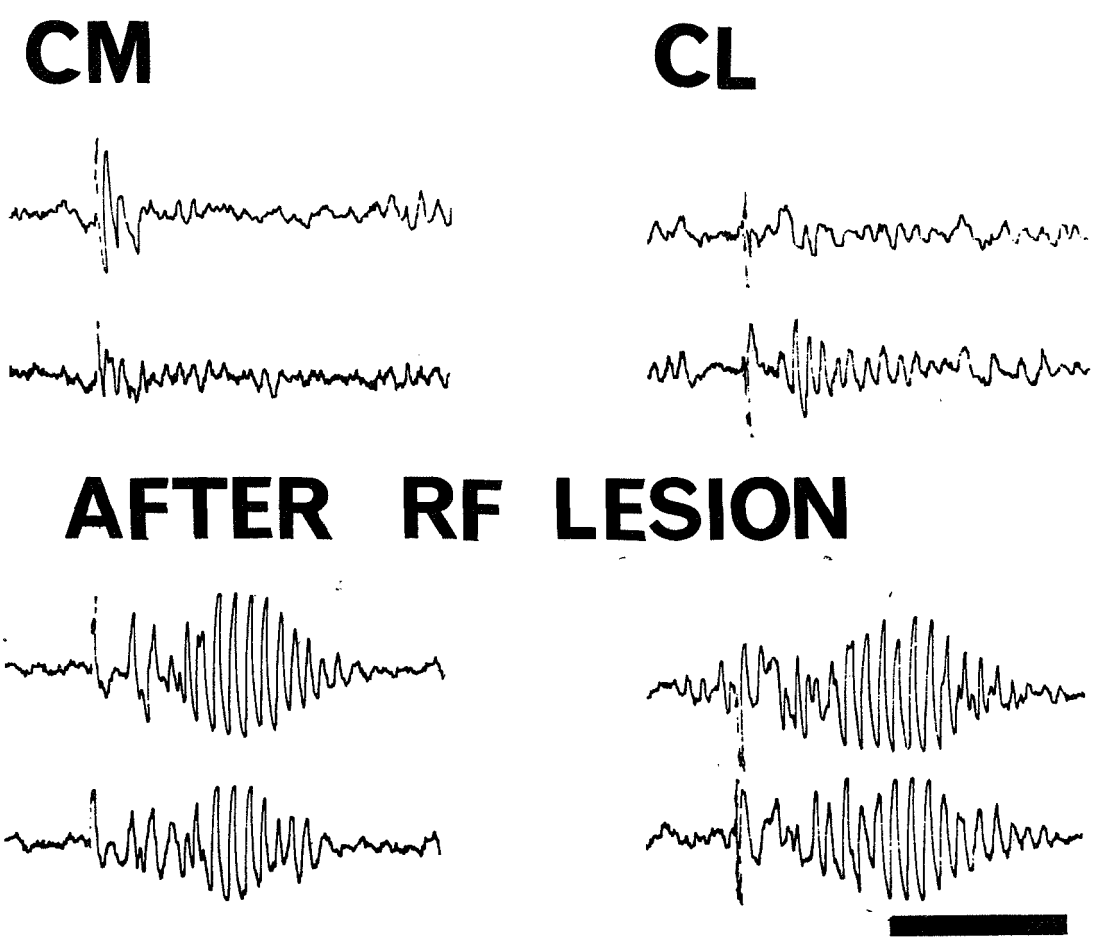

FIG. 3.— *Effect of reticular lesions on cortical afterdischarge. Upper row,* in the intact animal, cortical abortive afterdischarge of repetitive 8-12 c/sec waves induced by single shock (8V) applied to N. centrum medianum (CM) and N. centralis lateralis (CL) and recorded from anterior sigmoid gyrus (upper trace) and suprasylvian gyrus (lower trace). *Lower row,* after reticular lesion cortical afterdischarge appears of greater amplitude and duration. Calibration, 1 sec.

Cortical Responses to Repetitive Thalamic Stimulation.

As mentioned previously, in the intact animal, repetitive thalamic stimulation evoking cortical recruiting showed an initial incrementation (waxing) followed by a slight decrement (waning). Subsequent repetitive thalamic stimulation elicited cortical potentials of a constant amplitude (Fig. 4, A and C).

After reticular lesions (Fig. 4, B and D) recruiting showed an initial marked incrementation (waxing) followed by a marked decrement (waning). Cortical potentials to subsequent thalamic stimulation showed continuous variation in amplitude of waxing and waning nature. The amplitude of cortical re-

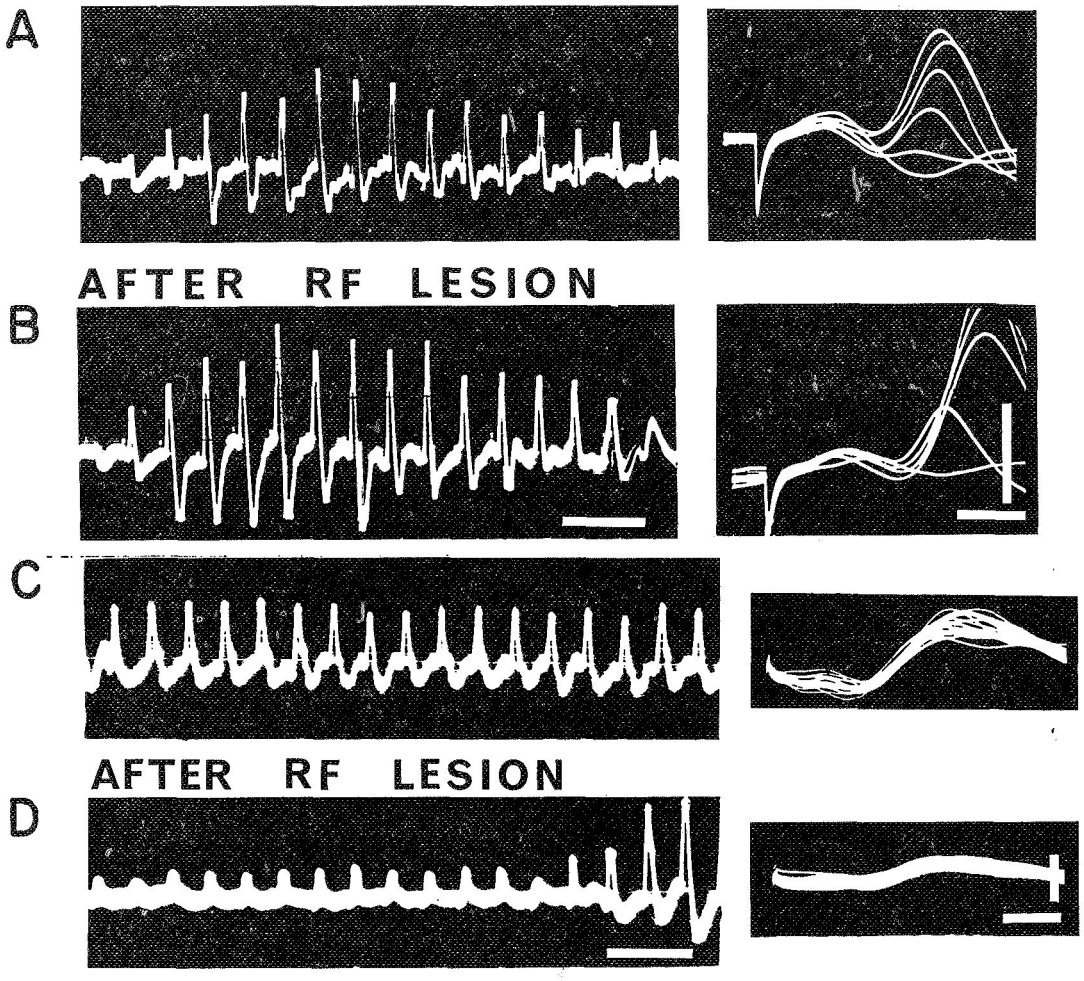

FIG. 4.—*Effect of reticular lesions on the amplitude of recruiting responses.* A, in the intact animal. Cortical recruiting responses elicited by NcM stimulation and recorded from anterior sigmoid gyrus. Recordings show the incremental (or waxing) period of the responses. B, following reticular lesions, same stimulation and recording as in A, shows faster development and larger amplitude during the incremental period. C, in the intact animal, cortical recruiting responses elicited by CL stimulation and recorded from suprasylvian gyrus. Recordings show the decremental (or waning) period of the responses. Notice that recruiting responses in an intact animal show slight decrementation with tendency to maintain constant amplitude. D, following reticular lesions, same stimulation and recording as in C, shows larger decrementation of the responses with tendency to increment again. Calibration, left 250 msec, 200 μV; right 10 msec, 200 μV.

cruiting was larger during the incremental period than during the decremental period in the intact animal.

The amplitude of cortical recruiting responses depended on the presence or absence of spontaneous spindle bursts. Maximal amplitude recruiting was observed when thalamic stimulation was presented at the end of a spontaneous spindle burst. Minimal or absent

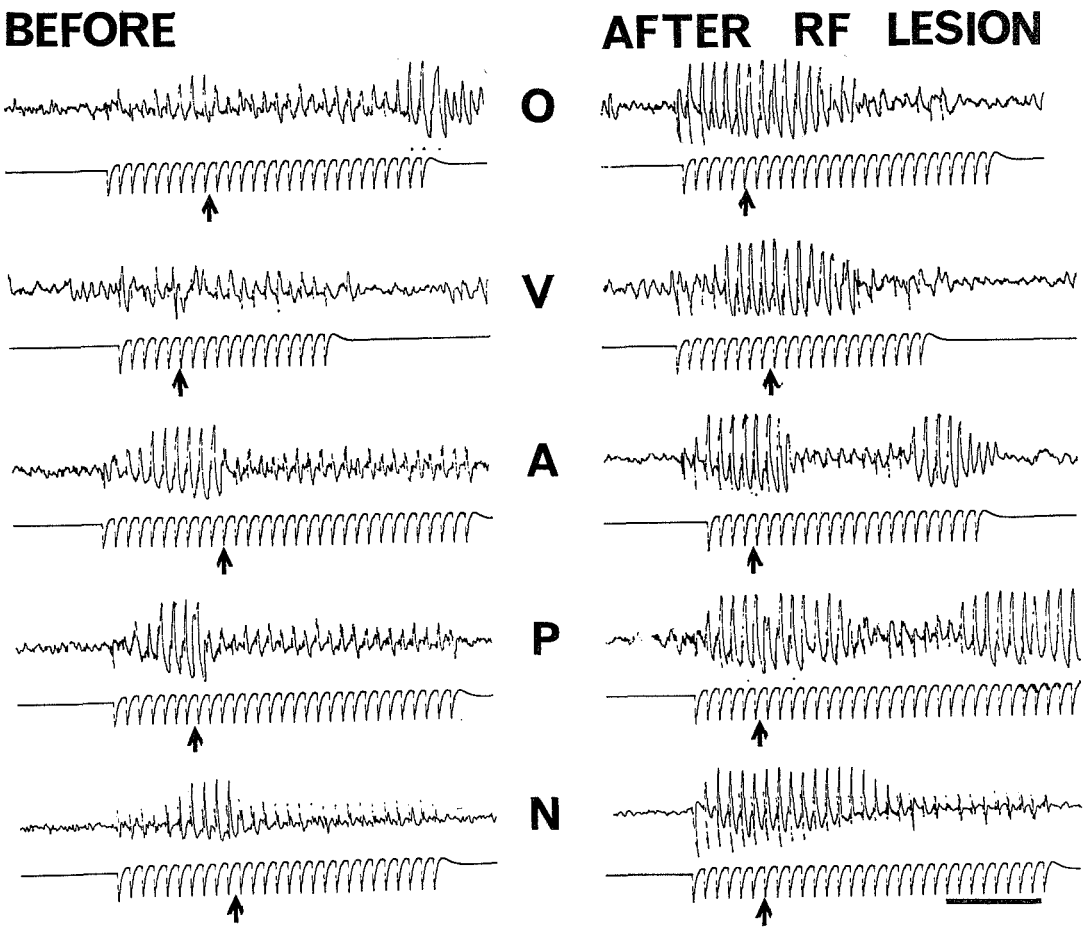

FIG. 5.— *Reticular lesions impair blocking of recruiting by sensory activation. Left,* in the intact animal. Cortical CM recruiting responses blocked by olfactory (O), visual (V), auditory (A) proprioceptive (P) and nociceptive (N) stimulation. Arrows indicate onset of the sensory activation. *Right,* after reticular lesions. Activation of the same sensory modalities failed to block recruiting responses. Calibration, 1 sec.

recruiting occurred when thalamic stimuli fell in the middle of a spindle burst.

Effect of Reticular Lesions on the Blocking of Recruiting Responses

Sensory Activation.

In these experiments, olfactory, visual, auditory, proprioceptive and nociceptive stimulation were introduced. In the case of visual, auditory and somesthetic stimulation primary cortical evoked responses were monitored before and after reticular lesions in lateral, ectosylvian and posterior sigmoid gyri.

Blocking of recruiting induced by sensory activation in the intact animal was impaired after reticular lesions (Fig. 5). This effect seemed to be independent of the arrival of afferent impulses in specific cortical areas, since

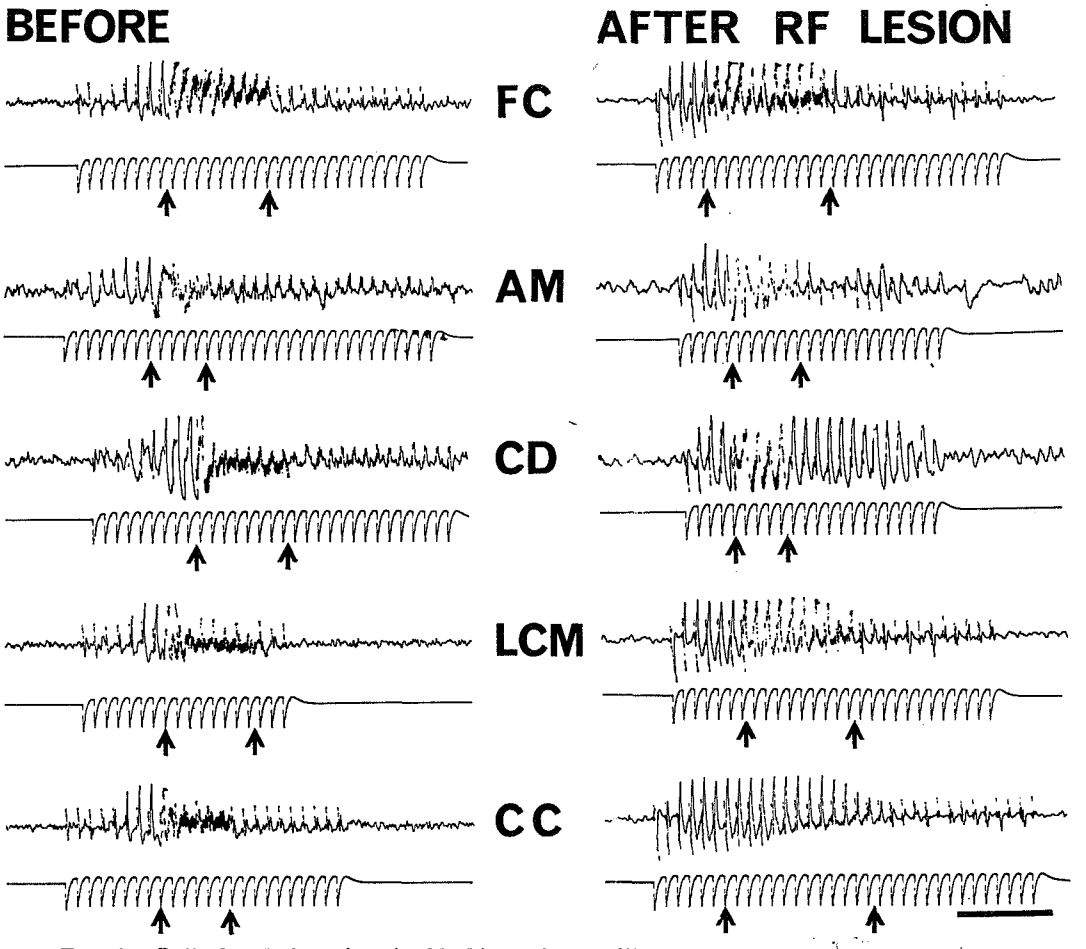

FIG. 6.—*Reticular lesions impair blocking of recruiting by brain electrical stimulation. Left,* in the intact animal. Cortical NCM recruiting blocked by high frequency stimulation of the frontal cortex (FC), amygdala (AM), caudate nucleus (CD), N. centrum medianum (LCM) and cerebral cortex (CC). Arrows indicate period of the high frequency stimulation. *Right,* after reticular lesions. Same stimulation failed to block recruiting responses. Calibration, 1 sec.

potentials evoked by sensory stimulation were still present. In addition, sensory activation failed to block spontaneous spindle bursts.

Central Electrical Stimulation.

In the intact animal, high frequency (150 c/sec) stimulation of the frontal cortex (gyrus proreus), the basolateral complex of the amygdala, the head of the caudate nucleus, N. centrum mediandum and the anterior-cerebellar cortex blocked recruiting responses (Fig. 6). In contrast, following reticular lesions, similar stimulation failed to block recruiting responses. Blocking of recruiting was still not observed using intensities 3 times greater than in the intact controls. Electrical stimulation of the above mentioned structures was also unable to block spontaneous spindle bursts.

Discussion

Tonic Reticular Influence Upon the Thalamocortical Synchronizing Systems

Our results provide further support of the idea that the mesencephalic reticular formation exerts a tonic inhibitory influence on thalamocortical synchronizing systems regulating spindle bursts and recruiting responses.[5, 8, 9, 10, 13] This tonic inhibitory reticular effect has been shown here by the fact that following reticular lesions thalamocortical synchrony was facilitated: presence of spontaneous spindle bursts, increase in amplitude and duration of thalamically induced afterdischarge and decrease in threshold and increased amplitude of cortical recruiting responses.

There are data, however, which indicate that this tonic reticular effect is not purely inhibitory, but is also regulatory of the synchronizing properties of a thalamo-orbitofrontal system which participates in amplitude modulation of cortical potentials.[20-25] (1) Following reticular lesions, spontaneous spindles, thalamically induced afterdischarges and recruiting responses were mainly facilitated over the frontal regions. (2) Cortical recruiting responses showed an important amplitude modulation, resulting in larger potentials during the incremental (waxing) part but smaller potentials during the decremental (waning) part. (3) The amplitude of thalamically induced afterdischarge and recruiting potentials depended largely on the occurrence of spontaneous spindle bursts, reflecting an intrinsic variation in the excitability of this thalamocortical system.

The Role of the Reticular Formation in Thalamocortical Activation

Moruzzi and Magoun,[13] and Lindsley *et al*,[10] have shown that ascending activating influences originating in infra-mesencephalic structures depend on the integrity of the brain stem reticular formation for manifesting their effect on thalamocortical synchrony. The role of the reticular formation in thalamocortical activation originating in supra-mesencephalic neural structures, has, however, remained unclear.

Previous investigations have shown that electrocortical activation could be elicited by high frequency stimulation of the frontal cortex,[2-4, 11, 12, 14, 19] the amygdala,[6] the caudate nucleus [18] and N. nucleus centrum medianum.[7, 17, 26] Electrocortical activation may be observed by stimulation of olfactory and visual sensory modalities terminating in supra-mesencephalic levels.[1, 16]

In studying the mechanism of thalamocortical activation of the above mentioned structures it was that neither cortical excision [2, 19] or section of the corpus callosum [3, 14] impaired cortically induced desynchronization.

In contrast, cortical activation was impaired following mesencephalic transection. [11, 12] Lesions in N. ventralis anterior of the thalamus, failed in preventing thalamocortical activation induced by either intralaminar thalamic [26] or caudate high frequency stim-

ulation.[18] In contrast, lesions in the posterior commisure interrupting thalamic influences upon the reticular formation, impaired thalamically induced desynchronization.[17, 26]

Arduini and Moruzzi [1] found that electrocortical activation produced by visual stimulation was abolished in the "cerveau isole" cat. Under the same conditions, olfactory stimulation was still able but only briefly, to block, cortical spindle bursts. This persistent olfactory activating effect after reticular lesions, may be explained on the basis of the level of the reticular transection, according to Rossi and Zirondoli,[16] and Roger *et al.*[15]

Velasco and Lindsley [21] analyzed thalamocortical activation by means of blocking of recruiting responses induced by activation of telencephalic, rhinencephalic, diencephalic, mesencephalic and rhombencephalic structures, as well as by olfactory, visual, auditory, proprioceptive and nociceptive sensory activation. In all cases, thalamocortical activation was qualitatively similar and consisted in a generalized suppression of the amplitude modulation of the cortical recruiting responses. It was postulated that thalamocortical activation is a unitary function mediated by a common reticular mechanism.

Present results demonstrate that central or peripheral neural activation, capable of blocking recruiting responses in the intact animal, fail to do so after lesions of the mesencephalic reticular formation at the level of the superior colliculi, suggesting that this critical area is essential for thalamocortical activation.

Supra-mesencephalic activating influences on thalamocortical systems would seem to require explanation on the basis of descending excitatory pathways to the mesencephalic reticular formation.

Summary

In twenty cats immobilized with gallamine, cortical recruiting responses and blocking of recruiting were studied comparatively before and after mesencephalic reticular lesions. Recruiting responses were elicited by repetitive (8/sec) stimulation of N. centrum medianum, N. centralis lateralis and N. centralis medialis.

Blocking of recruiting was produced by means of electrical stimulation of telencephalic, rhinencephalic, diencephalic and rhombencephalic structures, as well as by activation of various sensory modalities (visual, olfactory, auditory, proprioceptive and nociceptive). Electrolytic reticular lesions of the mesencephalic tegmentum were made at the level of the superior colliculi.

Following reticular lesions, cortical recruiting responses were different from those elicited in the intact animal. The main differences were: lower threshold, faster development and pronounced amplitude modulation, which resulted in larger amplitude of the responses during the waxing period and smaller during the waning period.

Thalamocortical activation, evaluated by blocking of recruiting responses, was abolished or seriously disrupted after mesencephalic reticular lesions, regardless of whether the blocking of recruiting was previously induced centrally or peripherally.

These results suggest that in an intact animal the reticular formation exerts a tonic regulatory effect on the thalamocortical mechanism responsible for the amplitude modulation of the cortical responses. In addition, it can be postulated that thalamocortical activation is a unitary function mediated by an essential reticular mechanism.

Resumen

La respuesta de reclutamiento cortical y el bloqueo del reclutamiento fueron estudiados comparativamente, en 20 gatos inmovilizados con gallamina, antes y después de lesiones reticulares. Las respuestas de reclutamiento se produjeron por

estimulación repetitiva (8/s) del núcleo centro mediano, núcleos centrales laterales y núcleos centralis medialis.

El bloqueo se produjo por medio de estimulación eléctrica de estructuras telencefálicas, rinencefálicas y romboencefálicas y activación de varias modalidades sensoriales (visual, olfatoria, auditiva, propioceptiva y nociceptiva). Se hicieron lesiones reticulares electrolíticas al nivel del colículo superior.

Las respuestas corticales de reclutamiento fueron diferentes después de lesión reticular de aquellas obtenidas en el animal intacto. Las diferencias principales eran: menor umbral, desarrollo más rápido y modulación pronunciada de amplitud que se evidenciaba en respuestas de mayor amplitud en el período de crecimiento y de menor amplitud en el de disminución. La activación talamocortical evaluada por el bloqueo de las respuestas de reclutamiento era abolida o seriamente desorganizada después de las lesiones reticulares mesencefálicas sin importar si el bloqueo de reclutamiento era previamente inducido central o periféricamente.

Estos resultados sugieren que en el animal intacto la formación reticulada ejerce un efecto tónico regulador en el mecanismo talamocortical responsable de la modulación de amplitud de la respuesta cortical. Además debe postularse que la activación talamocortical es una función unitaria, mediada por un mecanismo reticular esencial.

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